11.0 GUIDANCE FOR PERFORMING BIOLOGICAL EFFECTS TESTS

Biological effects tests, i.e., toxicity tests, may be necessary if Tier I evaluations conclude that the dredged material contains contaminants which might result in an unacceptable adverse impact to the benthic environment and/or the water column. Toxicity tests with whole sediment are used to determine the potential for effects on benthic (bottom dwelling) organisms; toxicity tests with suspensions/solutions of dredged material are conducted to determine the potential effects on water column organisms.

The objective of water column toxicity tests is to determine the potential impact of dissolved and suspended contaminants on organisms in the water column, after considering mixing. Test organisms should be representative of appropriately sensitive water column species existing in the vicinity of the disposal site.

The objective of benthic toxicity tests is to determine the potential impact of whole sediment on benthic organisms at and beyond the boundaries of the disposal site. The organisms used in testing should be representative of appropriately sensitive infaunal or epifaunal organisms existing in the vicinity of the disposal site. Benthic toxicity tests are intended to determine the potential chemical toxicity of a dredged material as distinct from its physical (e.g., grain-size) effects. Some organisms, particularly marine, are affected by differences in sediment textures or absence of sediments (McFarland, 1981; DeWitt et al., 1988). Control and reference sediments should be selected to minimize any artifactual effects of differences in grain size. If the sediment texture varies considerably between the dredged material and the control or reference sediments, any possible effects of grain size have to be determined and considered when designing the tests and evaluating the test results (e.g., DeWitt et al., 1988).

11.1 Tier III: Water Column Toxicity Tests

Tests to evaluate dredged-material impact on the water column involve exposing test organisms to an elutriate dilution series containing both dissolved and suspended components of the dredged material. The test organisms are added to the exposure chambers and exposed for a prescribed period (usually 96 h though some tests, e.g., bivalve larvae, may be run for shorter periods). The surviving organisms are examined at specified intervals and/or at the end of the test to determine if the test material is producing an effect. An introductory guide to general toxicity testing is presented in Part 8000 of APHA (1989) and in ASTM (1994b). Biological testing aspects of these reference publications may be followed as long as they do not conflict with this manual.

11.1.1 Species Selection

Three species are recommended for use in the water column exposure and should represent different phyla where possible (Table 11-1). The rationale for testing more than a single species is to cover the potential range of differing species sensitivities and to be environmentally protective. Of the species tested, at least one needs to be a sensitive benchmark (starred) species except as provided below; however, this does not preclude the use of more than one benchmark species. Those non-benchmark species listed in Table 11-1 or other species can be used if a summary of test conditions and test acceptability criteria similar to the starred benchmark species are established, and data from reference toxicity tests (see Appendix G.2.10.5.2) are provided on the sensitivity of the species. In order to be technically justfied, species proposed for use regionally and not listed in Table 11-1 would need to meet the species characteristics criteria, provided later in this Section, and proponents need to generate the following supporting information:

- data from toxicity tests using a set of reference chemicals with differing modes of action demonstrating that the proposed species is as sensitive or more sensitive than the species in Table 11-1

- summary of test conditions and test acceptability criteria.

If species proposed for use regionally are tested in conjunction with a benchmark species, the above supporting information is desirable but not needed. However, if the region substututes all species, the above information is needed.

The test organisms may be from healthy laboratory cultures or may be field collected, but not from within the influence of former or active disposal sites or other discharges. Ideally, the test species should be the same or closely related to those species that naturally dominate biological assemblages in the vicinity of the disposal site. Species characteristics to consider when designing water-column tests include, not in order of importance:

- readily available year-round
- tolerate handling and laboratory conditions
- give consistent, reproducible response to toxicants
- related phylogenetically and/or by ecological requirements to species characteristic of the water column of the disposal site area in the season of the proposed disposal
- standardized test protocols are available
- can be readily tested as juveniles or larvae to increase sensitivity
- important ecologically, economically, and/or recreationally
- appropriately sensitive.

Table 11-1. Candidate Toxicity Test Species for Determining Potential Water Column Impact of Dredged Material Disposal. Details of testing procedures are provided in Appendix E.

Crustaceans

Mysid shrimp, *Mysidopsis* sp.* (N)[d]
*Neomysis americana** (N)
*Holmesimysis costata** (N)
Grass shrimp, *Palaemonetes* sp. (N)
Commercial shrimp, *Penaeus* sp. (N)
Cladocerans, *Daphnia magna** (F) [d]
*Daphnia pulex** (F) [d]
*Ceriodaphnia dubia** (F)[d]

Fish

Silversides, *Menidia* sp.* (N) (E)[d]
Sheepshead minnow,
*Cyprinodon variegatus** (N)[d]
Speckled sanddab, *Citharicthys stigmaeus* (N)
Grunion, *Leuresthes tenuis* (N)
Fathead minnow, *Pimephales promelas** (F)[d]
Bluegill sunfish, *Lepomis macrochirus* (F)
Channel catfish, *Ictalurus punctatus* (F)
Rainbow trout, *Oncorhynchus mykiss** (F)

Bivalves

Larvae of
Oyster, *Crassostrea* sp.* (N,E)[a]
Mussel, *Mytilus edulis** (N,E)[a]

Echinoderms

Larvae of
Sea urchins, *Strongylocentrotus* sp.*[bc] (N)
Lytechinus pictus[b] (N)
Sanddollar, *Dendraster* sp.*[bc] (N)

Note: Examples are not presented in order of importance; however, the asterisks indicate sensitive recommended benchmark species. Benchmark species comprise a substantial data base, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species. Other species may be designated in future as benchmark species by EPA and USACE when the data on their response to contaminants are adequate.

[a] fertilized egg to hinged, D-shaped prodissoconch I larvae. Note that these two species can be used in estuarine waters down to appropriate low levels of salinity (see Appendix E).

[b] fertilized egg to pluteus larvae

[c] sperm fertilization

[d] These species can also be used in sublethal, chronic testing (methods for such testing are available but not detailed in this manual).

For the purpose of this manual, related to the tolerances of the test animals, (F) = Freshwater, salinity ≤ 1 ‰ (N) = Near Coastal, salinity ≥ 25‰ (E) = Estuarine, salinity 1-25‰. It is recognized that the commonly accepted salinity range for estuaries is 1-35‰ and near coastal salinity is usually greater than 30‰ salinity.

In addition to species occurring at the disposal site, other representative commercially available species or sensitive life stages of economically important species may be used. Mysids of the genera *Mysidopsis, Neomysis,* or *Holmesimysis* are highly recommended as test species. Embryo-larval stages of echinoderms, crustaceans, molluscs, or fish are also appropriate organisms. Adult fish and molluscs and large crustaceans must not be used for water column toxicity testing because of their generally greater resistance to contaminants, except as additional test organisms where data on economically important species are necessary to address public or regional concerns.

Regardless of their source, test organisms should be collected and handled as gently as possible. They should be gradually acclimated to the test conditions if test conditions differ from holding conditions. Field collected organisms must be tested within 2 weeks of collection. Animals from established laboratory cultures can be held indefinitely. Further details on methods are provided in ASTM (1994b).

11.1.2 Apparatus

Water column toxicity tests are generally conducted as static exposures in pre-cleaned glass chambers equipped with covers to minimize evaporation. The size of the chambers depends on the size of the test species. Before use, all glassware should be washed with detergent, rinsed five times with tap water, placed in a clean 10% HCl acid bath for a minimum of 4 h, rinsed with acetone, five times with tap water, and then thoroughly flushed with either distilled or deionized water.

Equipment and facilities must provide acceptable lighting requirements and temperature control. An environmental incubator or a water-bath system that allows temperature control within ±1°C is recommended.

11.1.3 Laboratory Conditions

Water column toxicity tests should be conducted under conditions known to be non-stressful to the test organisms. Salinity for marine/estuarine organisms should be stable within ±2‰ and, for all organisms, temperature should be stable within ±2°C throughout the exposure period. Dissolved-oxygen concentration should not be allowed to fall below an absolute minimum of 40% saturation for warm water species and 60% for cold water species. The temperature, salinity (if appropriate), dissolved oxygen, and pH in the test containers should be measured and recorded daily. Measurements of other parameters, for instance ammonia, may also be useful but need not be done daily.

11.1.4 Laboratory Procedures

Elutriate Preparation

Elutriate should be prepared using water collected from the dredging site. Disposal site water, clean seawater or freshwater, or artificial sea/salt mixtures should be used as dilution water for the tests. If sea/salt mixtures are used, they must be prepared in strict accordance with the manufacturer's instructions and allowed to age (with aeration) to ensure that all salts are in solution and pH has stabilized before use in any test. The elutriate is prepared by subsampling approximately 1 L of the homogenized dredged-material sample. The dredged material and unfiltered dredging site water are then combined in a sediment-to-water volumetric ratio of 1:4 at room temperature (22 ± 2°C). The mixture is then stirred vigorously for 30 min with a mechanical or magnetic stirrer. At 10 min intervals, the mixture is also stirred manually to ensure complete mixing. After the 30 min mixing period, the mixture is allowed to settle for 1 h. The liquid plus the material remaining in suspension after the settling period represents the 100% liquid plus suspended particulate phase. The supernatant is then carefully siphoned off, without disturbing the settled material, and immediately used for testing. With some very fine-grained dredged materials, it may be necessary to centrifuge the supernatant until the suspension is clear enough for the organisms to be visible in the testing chamber. Note that 15-40 L of elutriate may need to be prepared to test some species.

Test Design

The number of replicate exposure chambers per treatment should be determined according to the guidance in Appendix E. A minimum of five replicates per treatment and 10 organisms (except zooplankton or larvae) per replicate is generally recommended. Organism loading density must be low enough to avoid overcrowding stress.

At least three concentrations of the dredged-material elutriate should be tested; recommended treatments are 100%, 50%, and 10%. Water from the same source in which the animals were held prior to testing must be included as a control treatment subject to test survival acceptability criteria for controls (Appendix G). To properly evaluate the test results, any toxicity at 100% dilution water should also be determined.

The test organisms should be approximately of equal size and/or age and assigned randomly to the different treatments. Zooplankton and larvae are usually transferred with the aid of a pipette. Air must not be trapped on or under the animals during the transfer process. Larger animals may be transferred in fine-mesh nets. Animals which are dropped or exhibit abnormal behavior should be discarded.

The test chambers should be covered and randomly placed in an incubator or water bath. The test type is static non-renewal; the control and test solutions are not replaced. During the exposure period, aeration should not be supplied (unless necessary to keep dissolved oxygen concentration above 40% saturation

for warm water species or 60% for cold water species), and the test solutions should not be stirred. Some species of crustaceans, particularly larval forms, may require feeding during the test. All food used must be analyzed to ensure that it is acceptably free of contaminants and will support survival, growth or reproduction of test organisms (cf. EPA, 1994b).

Recommended test duration is 48-96 h for zooplankton and some larvae (e.g., oysters) and up to 96 h for other organisms. For bivalve larvae, the ASTM (1994c) procedure should be used. Useful procedures for other organisms are given in ASTM (1994b). For some tests, intermediate time observations may be made of survival but, for other tests, survival is only assessed at the end of the testing period. For intermediate observations, care must be taken to minimize any stress to the test organisms. Only the number of living organisms are counted, not the number of dead. An animal is judged dead if it does not move either after the water is gently swirled or after a sensitive part of its body is gently touched with a probe. At intermediate observations, a pipette or forceps is used to remove dead organisms, molted exoskeletons, and food debris.

If greater than acceptable mean mortality or abnormal development occurs in the control as defined in the procedures for proper conduct of that test, the test must be repeated. Further QA/QC considerations are provided in Appendix G.

11.1.5 Data Presentation and Analysis

Data Presentation

The data for each test species should be presented in separate tables that include the following information:

- the scientific name of the test species
- the number of organisms in each treatment at the start of the test
- the number of organisms alive at each observation period, if applicable
- the number of organisms recovered alive and/or in normal health from each chamber at the end of the test
- additional information including water quality and any behavioral or other abnormalities.

Data Analysis

It is possible that no mortality or other effects will be observed in any of the treatments or that survival or other effects in the dredged material treatments will be equal to or higher than in the control or in the dilution water treatments. In either of these situations, there is no need for statistical analysis and no indication of water column toxicity attributable to the dredged material. However, if survival or other

effects in the dilution water treatment is at least 10% greater than the 100% dredged-material treatment, the data have to be evaluated statistically to determine whether the dredged-material suspension is significantly more toxic than the dilution water. If the 100% dredged-material treatment is not statistically different from the dilution water, the dredged material is predicted not to be acutely toxic to water column organisms. An LC_{50} should not be calculated unless at least 50% of the test organisms die in at least one of the serial dilutions . If there are no mortalities greater than 50%, then the LC_{50} is assumed to be ≥100%. If a statistical difference exists and greater than 50% mortality or other effects occur in all of the treatments, it is not possible to calculate an LC_{50} or EC_{50} value. If the conditions are highly toxic, such that the 10% treatment has greater than 50% mortality, further dilution must be made (new treatments of less than 10% dredged material) to attain a survival of greater than 50% and determine the LC_{50} or EC_{50} by interpolation. Statistical procedures recommended for analyzing the test data are described in detail in Appendix D.

11.1.6 Conclusions

The Tier III water-column effects evaluation involves using a numerical model comparison with the WQS. Descriptions of the models and applications are given in Appendix C, and the models are provided on the diskettes that can be found in the pocket inside the back cover of this manual.

The modeled concentrations of the dredged material (expressed as percentages) are compared to 0.01 of the 48- or 96-h LC_{50} or EC_{50}, depending on the test duration. The maximum allowable concentration outside the mixing zone is 0.01 LC_{50} or EC_{50}. Note that the 0.01 factor is intended for acute mortality data (e.g., relating acute to chronic toxicity) and not for more subtle effects such as abnormalities, growth or reproduction, including EC_{50} data (NAS, 1972). However, in the absence of other alternatives, the 0.01 application factor should be applied to EC_{50} data although it is recognized that these results will be conservative and that derivation of this historic application factor was largely a matter of "best professional judgement" by the NAS (1972). Thus, site-specific review may be required in some cases to determine compliance.

11.2 Tier III: Benthic Toxicity Tests

Toxicity tests with whole sediment are designed to determine whether the dredged material is likely to produce unacceptable adverse effects on benthic organisms. In benthic toxicity tests, the test animals are exposed to the whole sediment and any effects recorded.

11.2.1 Species Selection

Species representing three life history strategies are recommended for use in the whole sediment toxicity tests, one each representing a filter feeder, deposit feeder and a burrowing organism where possible (Table 11-2). The rationale for testing more than a single species is to cover the range of differing species sensitivities and to be environmentally protective. No single species is adequately protective of the broad range of possible chemical contaminants nor of the equally broad range of possible biological responses. Of the species tested, at least one sensitive benchmark (starred) species needs to be be used in all cases except as provided below; however, this does not preclude the use of benchmark species representative of all three required categories. If only two different species are being tested they should, together, cover the following three life history strategies: filter feeder, deposit feeder, burrower. Since amphipods are excellent organisms for short term toxicity, they are recommended as one of the species to be tested. Non-benchmark species listed in Table 11-2 can be used if a summary of test conditions and test acceptability criteria similar to the starred benchmark species are established and data from reference toxicity tests (see Appendix G.2.10.5.2) are provided on the sensitivity of the species. In order be technically justified, species proposed for use regionally and not listed in Table 11-2 need to meet the species characteristics criteria provided later in this section and proponents need to provide the following supporting information:

- data from toxicity tests using a set of reference chemicals with differing modes of action demonstrating that the proposed species is as sensitive or more sensitive than the species in Table 11-2

- summary of test conditions and test acceptability criteria.

If species proposed for use regionally are tested in conjunction with a benchmark species, the above supporting information is desirable but not required. However, if the region substitutes all species, the above information is needed.

Benthic organisms are used to evaluate the potential benthic impact of dredged material disposal. Testing of contaminated sediments (e.g., Word et al., 1989; Gentile et al., 1988; Rogerson et al., 1985) and regulatory program experience since 1977 under the Marine Protection, Research, and Sanctuaries Act and the Clean Water Act have shown that different species have various degrees of sensitivity to the physical and chemical composition of sediments.

To accurately evaluate potential benthic impact, appropriately sensitive toxicity test species should be related as closely as possible, both phylogenetically and ecologically, to benthic organisms in the disposal

Table 11-2. Candidate Acute Toxicity Test Species for Determining Potential Benthic Impact of Dredged-Material Disposal. Details of testing procedures are provided in Appendix E. Additional guidance is provided in ASTM (1994d,e,f,g) and EPA (1994c,d).

Amphipod Crustaceans

*Ampelisca abdita** (N)[a] [d,b]
*Rhepoxynius abronius** (N) [d,b]
Grandidierella japonica (N) [d,b]
Corophium sp. (N) [f,d,b]
*Leptocheirus plumulosus** (E,N)[a] [d,b]
*Eohaustorius estuarius** (E) [d,b]
*Hyalella azteca** (E,F)[a] [d,b]

Polychaetes

Neanthes arenaceodentata (N)[a] [d,b]

Juvenile Bivalves (clams)

Paper pondshell freshwater mussel, *Anodonta imbecillis* (F) [f,b]

Crustaceans other than Amphipods

Mysid shrimp, *Mysidopsis* sp. (N) [f,d]
Neomysis americana (N) [f]
Holmesimysis costata (N) [f]
Commercial shrimp, *Penaeus* sp. (N) [d,b]
Grass shrimp, *Palaemonetes* sp. (N,E)[b] [d]

Insect Larvae

Midges, *Chironomus tentans** (F)[a] [d,b]
*C. riparius** (F)[a] [d,b]
Mayfly, *Hexagenia limbata* (F) [d,b]

Oligochaetes

Pristina leidyi (F) [d,b]
Tubifex tubifex (F)[a] [d,b]
Lumbriculus variegatus (F)[a] [d,b]

Note: Examples are not presented in order of importance; however, the asterisks indicate sensitive recommended benchmark species. Benchmark species comprise a substantial data base, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species. Other species may be designated in future as benchmark species by EPA and the USACE when the data on their response to contaminants are adequate. Only benthic species should be tested. Although sediment dwellers are preferable, intimate contact with sediment is acceptable. Note that testing with all recommended taxa is not required; however, at least one starred amphipod taxon must be tested.

[f = filter feeder; d = deposit feeder; b = burrower]. Note that *A. abdita*, *L. plumulosus*, *C. tentans*, and *H. limbata* are not direct filter feeders, but are suspension feeders.

[a] These species can also be used in sublethal, chronic testing (methods for such testing are available but not detailed in this manual).

[b] This species can be used in estuarine waters down to appropriate low levels of salinity (see Appendix E).

For the purposes of this manual, related to the tolerances of the test animals, (F) = Freshwater, salinity $\leq$ 1‰ (N) = Near Coastal, salinity $\geq$ 25‰ (E) = Estuarine, salinity 1-25‰. It is recognized that the commonly accepted salinity range for estuaries is 1-35‰ and near coastal water is usually greater than 30‰ salinity.

site area. Commercially important but possibly less sensitive benthic species in the vicinity of the disposal site may also be considered for testing.

Sediment grain size is likely to vary substantially between the dredged material, the reference sediment, and the control sediment. If candidate test species are overly sensitive to the different grain sizes (for instance, excessive mortality in the reference sediments attributable to grain size and not to other factors), either this must be taken into account (e.g., DeWitt et al., 1988) or other, more grain-size tolerant species should be considered for the project.

Final selection of test species for a particular dredged material disposal project should be made in consultation with regional regulatory and scientific personnel. Two phylogenetically and ecologically different species are recommended to account for different sensitivities to contaminants. The following is a list, not necessarily in order of importance, of characteristics to consider for species selection:

- readily available year-round
- preferably ingest sediments
- tolerate grain sizes of dredged material and control and reference sediments equally well or differences should be accounted for
- give consistent, reproducible response to toxicants
- tolerate handling and laboratory conditions
- related phylogenetically and/or by ecological requirements to species characteristic of the benthic environment of the disposal site area in the season of the proposed disposal
- standardized test protocols are available
- important ecologically, economically, and/or recreationally
- appropriately sensitive.

Infaunal amphipods are excellent organisms for short term toxicity tests with whole sediment (Swartz et al., 1979, 1985; Mearns and Word, 1982; Rogerson et al., 1985; Nebeker et al., 1984; Gentile et al., 1988; Scott and Redmond, 1989; Word et al., 1989; Burton, 1991), and are strongly recommended as appropriate test species for acute toxicity bioassays in marine/estuarine/fresh waters. Guidance on available testing procedures (static, 10-d exposures) provided in ASTM (1994d,e) may be followed on all points that do not conflict with this manual. Infaunal amphipods are:

- sensitive
- readily available
- as a group, tolerant of a wide range of grain sizes and laboratory exposure conditions
- ecologically relevant to most dredged material disposal sites.

The identity of all species should be verified by experienced taxonomists, particularly for animals collected in the field. If the toxicity test animals are also to be used in estimating bioaccumulation potential, the factors discussed in Section 12.1.1 for species selection should also be considered.

11.2.2 Laboratory Procedures

General Test Procedures

Acceptable water quality parameters during testing include but are not necessarily restricted to:

- the correct temperature and pH range
- adequate oxygen levels
- proper lighting
- the correct salinity range (near coastal and estuarine organisms)
- the correct hardness range (fresh water organisms)
- the absence of, or insignificant concentrations of, toxicants such as ammonia.

Amphipod and other small organism tests are often, but not always, conducted in 1 L containers under static conditions (Appendix E). Static renewal or even flow-through methods such as those described by Redmond et al. (1989) or Benoit et al. (1993) may be required for certain tests or where static non-renewal conditions would result in unacceptable build-up of, for instance, ammonia and/or sulfides (see second and third paragraphs, Ammonia and Sulfide toxicity, this section).

Before use, all glassware should be washed with detergent, rinsed with acetone, five times with tap water, placed in a clean 10% HCl acid bath for a minimum of 4 h, rinsed five times with tap water, and then thoroughly flushed with either distilled or deionized water. Equipment and facilities must provide acceptable lighting requirements and temperature control. An environmental incubator or a water-bath system that allows temperature control within ± 1°C is recommended.

Dilution water should not be stressful to the test organisms, and should be stable throughout the exposure period. Salinity for marine/estuarine organisms should be stable within ± 2‰ and, for all organisms, temperature should be stable within ± 2°C throughout the exposure period. Dissolved oxygen concentration should not be allowed to fall below an absolute minimum of 40% saturation for warm water species and 60% for cold water species. The flow to the exposure chamber should be directed to achieve good mixing without disturbing the sediment on the bottom of the chamber.

A minimum of five replicate exposure chambers for the dredged material, reference, and control is recommended. The standard test duration is 10 d.

The quantity of sediment needed depends on the size of the exposure chambers. The sediment should be deep enough to meet the biological needs of the test organisms, i.e., allow organisms to burrow in their normal position, etc. Overcrowding of organisms must be avoided.

Prior to use in toxicity tests, sediments must be thoroughly homogenized. Very small amounts of clean diluent water may be added to facilitate mixing. If separation into liquid and solid phases occurs in posthomogenization storage, remixing will be required prior to usage.

The reference and control sediments, as well as the dredged material being tested, may contain live organisms. If necessary, macrobenthic organisms can be removed by press-sieving the sediments through an appropriately sized screen immediately prior to testing. The material remaining on the screen should be noted and discarded.

The experimental procedure described in ASTM (1994d) should be followed for preparing the exposure chambers for amphipod toxicity tests. For larger exposure chambers, sediment should be placed on the bottom of the exposure chamber and covered with clean diluent water; any sediment suspended during placement should be allowed to settle for 24 h before introducing the test organisms. In continuous-flow tests, the flow should be established after most of the suspended sediment has settled, usually 12 to 24 h, but at least 1 h before introducing the test organisms.

During the exposure period, daily records should be kept of obvious mortalities, emergence of infaunal organisms, formation of tubes or burrows, and any other or unusual behavior. Daily records of water quality (e.g., dissolved oxygen, salinity (if appropriate), ammonia, temperature, pH) should be maintained using test containers appropriate for this purpose. In flow-through or static-renewal systems, water quality may be kept within acceptable bounds by increasing the flow rate or frequency of water changes.

After the exposure period, live organisms are removed to clean diluent water, which may include sieving the sediments, and then counted. If greater than acceptable mean mortality occurs in the control, as defined in the procedures for proper conduct of that test, the test must be repeated. Organisms which show any response to gentle probing of sensitive parts or gentle swirling of the water should be considered alive. Sediment dwellers (e.g., amphipods) not recovered at the end of the test have to be considered dead. If organisms from these toxicity tests are to be used in estimating bioaccumulation potential, the survivors are gently and rapidly counted and then treated as described in Section 12.

Ammonia and Sulfide Toxicity

Whether ammonia is or is not a contaminant of concern depends on the disposal site. In order to identify elutriate or solid phase dredged material toxicity due to ammonia, it is essential to make routine measurements of ammonia on appropriate test fractions. These measurements are compared to water-only toxicity data for the same species used in the dredged material test (see Appendix F). The water-only toxicity data generated separately should be generated under conditions (e.g., pH, test length) reasonably similar to those in the test with the dredged material. If ammonia concentrations are too low to have potentially caused the observed toxicity in the dredged material sample, other contaminants are responsible for the toxicity. If ammonia concentrations are high enough to have caused the observed toxicity, toxicity identification evaluation (TIE) procedures should be used to confirm this suspicion. When there is no TIE confirmation that ammonia is responsible for sediment toxicity, it must be assumed that persistent contaminants other than ammonia are causing toxicity. Full details of procedures to identify ammonia as a toxicant in toxicity tests with dredged material are provided in Appendix F.

Whenever chemical evidence of ammonia is present at toxicologically important levels, i.e. ammonia concentrations exceed the species-specific acceptability ranges shown below (or 20 mg/L for freshwater organisms), and ammonia is not a contaminant of concern at the disposal site, the laboratory analyst should set up one or more beakers explicitly for the purpose of measuring interstitial ammonia. Ammonia in the sediment interstitial water should be reduced to below the species-specific level shown below (or to below 20 mg/L for freshwater organisms) before adding the benthic test organisms. Ammonia concentrations in the interstitial water can be reduced by sufficiently aerating the sample at saturation and replacing two volumes of water per day. The analyst should measure interstitial ammonia each day until it reaches a concentration below the appropriate species-specific level (or ≤20 mg/L for freshwater organisms). After placing the test organisms in the sediment, the analyst should ensure that ammonia concentrations remain within an acceptable range by conducting the toxicity test with continuous flow or volume replacement not to exceed two volumes per day. Peer-reviewed papers that deal with ammonia in sediments include: Dewitt et al. (1988), Scott and Redmond (1989), Burton (1991), EPA (1992, 1994c, 1994d), Benoit et al. (1993), Ankley et al. (1991, 1992a, 1992c, 1994).

General Acceptability Ranges for Ammonia in Marine and Estuarine Amphipod Sediment Toxicity Tests.

Parameter	*Rhepoxynius*	*Ampelisca*	*Eohaustorius*	*Leptocheirus*
Ammonia (total mg/L, pH 7.7)	<30	<30	<60	<60
Ammonia (unionized mg/L, pH 7.7)	<0.4	<0.4	<0.8	<0.8

The chemistry and toxicology of sulfides is less well-understood than that of ammonia. However, sulfides are not likely to be a problem in most open-water situations, or in bioassays where adequate oxygen levels are maintained in the overlying water.

11.2.3 Chronic/Sublethal Tests

Chronic/sublethal responses to sediment are presently only available, in addition to the end-point of survival, for a very few toxicity tests, for example: the amphipods *Hyalella azteca*, *Ampelisca abdita* and *Leptocheirus plumulosus;* the midges *Chironomus tentans* and *C. riparius*; the oligochaetes *Tubifex tubifex* and *Lumbriculus variegatus,* and the polychaete *Neanthes arenaceodentata.* [Note: EPA has recently developed chronic sediment toxicity test methods for freshwater organisms (*C. tentans and H. azteca*). EPA and USACE are jointly developing a chronic sediment toxicity test method manual for marine and estuarine organisms (*L. plumulosus*). These documents are currently under review and will be published as standard methods manuals.] Unlike acute toxicity tests, there is presently no consensus as to what level of chronic/sublethal effects (e.g., reduction of growth, reproduction, fecundity, survival of young) is cause for concern. Further, there is also no consensus as to when such effects would preclude disposal or would constitute unacceptable adverse effects requiring some type of management action. Hence, chronic/sublethal tests are not presently part of Tier III in this national manual. However, regional testing manuals may apply appropriate chronic/sublethal tests to sediments in advance of their inclusion in this national manual provided this is done with a benchmark species (e.g., *C. tentans*) or *in addition to* the benchmark testing.

Guidance for conducting the above tests may be found in publications including Nebeker and Miller (1988), Nebeker et al. (1984), Johns and Ginn (1990), Johns et al. (1990), Ingersoll and Nelson (1990), Dillon et al. (1993), Phipps et al. (1993), McGee et al. (1993). Burton (1991) provides a comprehensive review of freshwater sediment toxicity tests. Survival and growth are the endpoints of all of these tests. In addition, some tests also measure reproductive end-points.

Criteria for control acceptability for chronic/sublethal tests are specific to the test and organism. If control criteria are exceeded, the test must be repeated.

11.2.4 Data Presentation and Analysis

Data Presentation

The data for each test species should be presented in separate tables that include the following information:

- scientific name of the test species

- number of organisms in each treatment at the start of the test
- number of organisms recovered alive and/or in normal health from each chamber at the end of the test (including positive and negative controls)
- information regarding emergence, burrowing, tube building, behavioral abnormalities, growth, reproduction, and any other observations
- water-quality data for each test chamber for each day.

Data Analysis

It is possible that neither mortality nor other effects will be observed in any of the treatments or that survival in the dredged material will be equal to or higher than survival in the reference or control sediments. In either of these situations, there is no need for statistical analysis and no indication of adverse effects due to the dredged material. Similarly, if survival is higher in test sediments than in the control, but lower than in the reference area, and control survival is at acceptable levels (i.e., 90% or greater survival), there is no need for statistical analysis and no indication of benthic toxicity due to the dredged material. However, if survival in the reference sediment is higher than in the dredged material treatments and exceeds the allowable percent difference between the two treatments, the data have to be analyzed statistically to determine whether there is a significant difference between the reference and dredged material. Statistical procedures recommended for analyzing benthic acute toxicity data are described in detail in Appendix D. Local guidance must be developed to interpret chronic/sublethal tests.

11.2.5 Conclusions

Guidance on the use of the results to reach a determination is provided in Section 6.2.

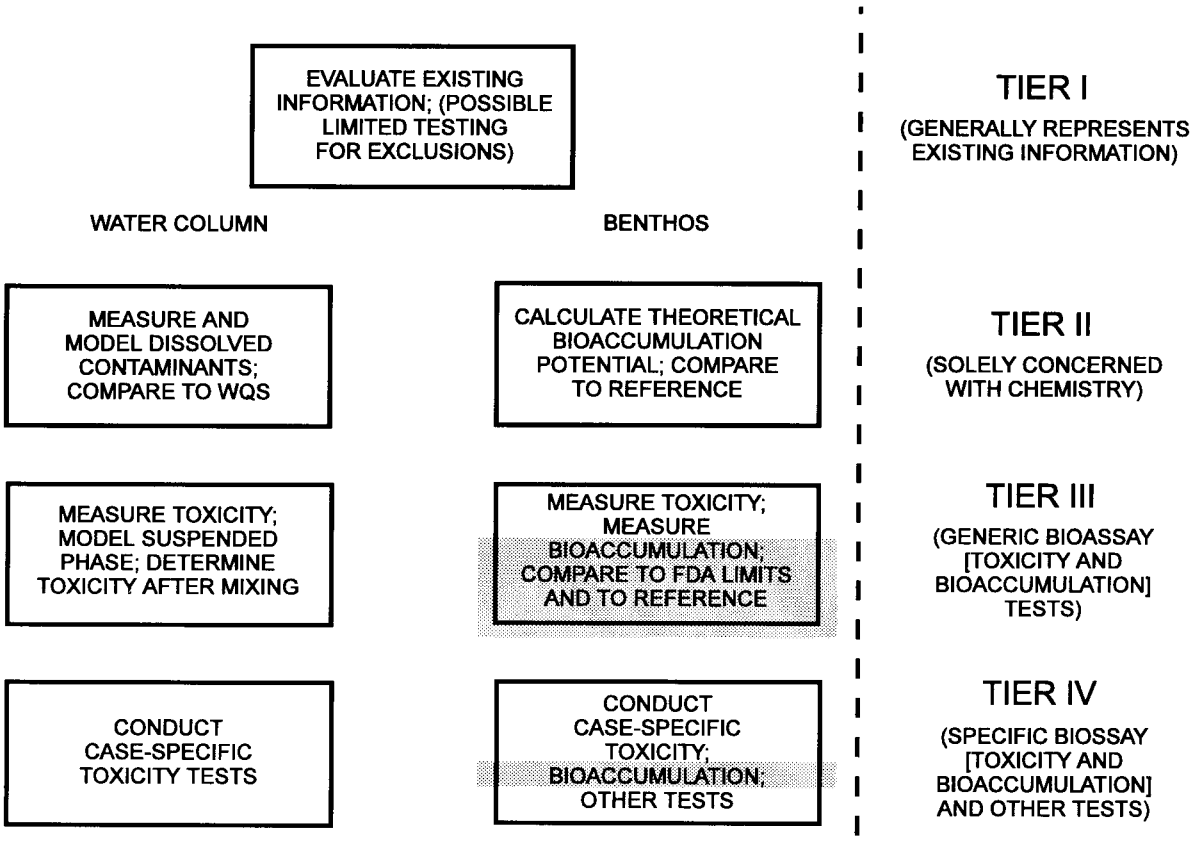
11.3 Tier IV: Chronic/Sublethal Effects Evaluations

At present, it is not appropriate to incorporate sediment chronic/sublethal effects testing in this national manual (see Sections 6.0 and 11.2.3). When standardized chronic effects tests are approved, they will be incorporated in Tier III. Until then, such non-standard tests should be used in Tier IV except where regional testing manuals apply such tests in advance of their inclusion in future revisions of this national manual, provided this is done with a benchmark species or *in addition to* the benchmark testing.

11.4 Tier IV: Case Specific Evaluations

Biological effects tests in Tier IV should be used only in situations that warrant special investigative procedures. They may include chronic/sublethal tests, field studies such as benthic infaunal studies (EPA, 1992), experimental studies such as *in situ* toxicity tests or toxicity identification evaluation (Ankley et

al., 1992a), risk assessments and/or no effects levels for aquatic life. In such cases, test procedures have to be tailored for specific situations, and general guidance cannot be offered. Such studies have to be selected, designed, and evaluated as the need arises, with the assistance of administrative and scientific expertise from EPA and USACE, and other sources as appropriate.


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)